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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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60/067,357 2 December 1997 (02.12.97) US

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10 September 1999 (10.09.99)

(54) Title: NON–TOXIC IMMUNE STIMULATING ENTEROTOXIN COMPOSITIONS

(57) Abstract

Pyrogenic toxins, such as staphylococcal enterotoxins, modified in the disulfide loop region are provided. The modified toxins retain useful biological properties but have substantially reduced toxicity compared to the corresponding unmodified native toxin. The native pyrogenic toxins are typically modified by deletions within the disulfide loop region to produce modified enterotoxins having 100–fold or greater decrease in toxicity.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/25107

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/00; C07H 21/04; C07K 1/00, 2/00
US.CL :530/300, 350; 536/23.7

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/300, 350; 536/23.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, BIOSIS, CA, EMBASE
terms: pyrogenic, entertoxin, modified, fragment, staphylococcus, streptococcus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97/36932 A1 (PHARMACIA & UPJOHN AB) 09 OCTOBER 1997 (09/10/97), see entire document.	1-26
Y	NISHI et al. B Cell Epitope Mapping of the Bacterial Superantigen Staphylococcal Enterotoxin B. Journal of Immunology. 01 January 1997, Vol. 158, No. 1, pages 247-254.	1-26
Y	ALAKHOV et al. Identification of functionally active fragments of staphylococcal enterotoxin B. Eur. J. Biochem. 01 November 1992, Vol. 209, pages 823-828.	1-26

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 14 JUNE 1999	Date of mailing of the international search report 08 JUL 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MARK NAVARRO Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/25107

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BINEK et al. Localisation of the mitogenic epitope of staphylococcal enterotoxin B. J. Med. Microbiol. March 1992, Vol. 36, pages 156-163.	1-26

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 06 September 1999 (06.09.99)	
International application No. PCT/US98/25107	**Applicant's or agent's file reference** 12136.1WO01
International filing date (day/month/year) 01 December 1998 (01.12.98)	**Priority date** (day/month/year) 02 December 1997 (02.12.97)
Applicant BOHACH, Gregory, I.	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

28 June 1999 (28.06.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Jean-Marie McAdams
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 12136.WO01	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US98/25107	International filing date *(day/month/year)* 01 DECEMBER 1998	Priority date *(day/month/year)* 02 DECEMBER 1997
International Patent Classification (IPC) or national classification and IPC IPC(7): A61K 38/00; C07H 21/04; C07K 1/00, 2/00 and US Cl.: 530/300, 350; 536/23.7		
Applicant IDAHO RESEARCH FOUNDATION, INC.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand 28 JUNE 1999	Date of completion of this report 03 APRIL 2000
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MARK NAVARRO Telephone No. (703) 308-0196

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US98/25107

I. Basis of the report

1. With regard to the **elements** of the international application: *

[X] the international application as originally filed

[X] the description:
pages 1-20 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the claims:
pages 21-23 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the drawings:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the sequence listing part of the description:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US98/25107

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	1-26	YES
	Claims	NONE	NO
Inventive Step (IS)	Claims	NONE	YES
	Claims	1-26	NO
Industrial Applicability (IA)	Claims	1-26	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1-26 lack an inventive step under PCT Article 33(3) as being obvious over Pharmacia & Upjohn AB in view of Alakhov et al and Nishi et al.

Applicant's are asserting that a simple teaching that residues in a superantigen can be replaced (Pharmacia Upjohn) combined with disclosure that a cysteine loop in the superantigen is sensitive to proteolytic cleavage and a disclosure of a dominant epitope in the superantigen does not teach or suggest that modifying the disulfide loop would produce a modified superantigen which retains biological activity but demonstrates significantly lower toxicity. Applicant's arguments have been fully considered but are not found to be fully persuasive.

Applicant's arguments are not found to be fully persuasive in view of the teaching of Alakhov et al, which set forth that staphylococcal enterotoxin B contains a proteolysis-sensitive sequence in the cysteine loop formed by two half-cystines located in the middle of the toxin polypeptide chain. Alakhov et al further set forth that the toxin's C-terminal fragment, which contains the cysteine loop, possesses an ability to activate calmodulin-dependent enzymes and is probably the toxicogenic part of the enterotoxin. (See abstract). Consequently one of skill in the art at the time of the invention, would have been motivated to replace amino acid residues within a superantigen as set forth by Pharmacia Upjohn within the cysteine loop region of staphylococcal enterotoxin B, in view that Alakhov et al have set forth that this region is responsible for the ability to activate calmodulin-dependent enzymes and is probably the toxicogenic part of the enterotoxin.

The claims are directed to a modified pyrogenic toxin derived from a native disulfide loop-containing pyrogenic toxin, wherein the modified toxin comprises a disulfide loop region containing no more than 10 amino acid residues.

Pharmacia & Upjohn AB (WO 97/36932) teach of modified superantigen (SA I) in which an amino acid residue in a superantigen region (region I) determining binding to TCR and T cell activation have been replaced by another amino acid residue while retaining the ability to activate a subset of T cells. (See abstract). (Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):

Pharmacia & Upjohn AB do not teach of a modified toxin which comprises a disulfide loop region containing no more than 10 amino acid residues.

Alakhov et al teach that staphylococcal enterotoxin B contains a proteolysis-sensitive sequence in the cysteine loop formed by two half-cysteines located in the middle of the toxin polypeptide chain. Alakhov et al further teach that the N-terminal fragment of *staphylococcal* enterotoxin B is capable of activating T cell proliferation in the culture of human mononuclear cells practically to the same degree as the intact enterotoxin. (See abstract).

Nishi et al teach that amino acids 225-234 of the bacterial superantigen *Staphylococcal* enterotoxin B is the dominant B cell epitope. (See abstract).

It would have been obvious to one of ordinary skill in the art at the time of the invention to have incorporated the method of generating modified superantigens as taught by Pharmacia & Upjohn AB and to further introduce those mutations in the cysteine loop of *staphylococcal* enterotoxin B, in view that amino acids 225-234 of *staphyloccal* enterotoxin B are the dominant B cell epitope. One would have been motivated to create a mutant in the superantigen near this location in view of the teaching of Nishi et al that amino acids 225-234 are the dominant B cell epitope.

---------- NEW CITATIONS ----------

NONE

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A2

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(54) Title: NON–TOXIC IMMUNE STIMULATING ENTEROTOXIN COMPOSITIONS

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